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PRITCHARDIA BOLIVIENSIS N. GEN., N. SP. (ANOPLOCEPHALIDAE: LINSTOWINAE), A TAPEWORM FROM OPOSSUMS (DIDELPHIDAE) IN THE YUNGAS AND LOWLANDS OF BOLIVIA AND ATLANTIC FOREST OF PARAGUAY

SCOTT L. GARDNER, F. AGUSTÍN JIMÉNEZ, AND MARIEL L. CAMPBELL

ABSTRACT

Pritchardia boliviensis n. gen. n. sp. (Anoplocephalidae: Linstowiinae) is described from marsupials (*Marmosops noctivagus*, *Metachirus nudicaudatus*, *Gracilinanus* sp.) collected in Bolivia and Paraguay. These cestodes have a very small strobila with only three segments, regularly alternating genital pores, genital ducts crossing excretory canals ventrally, ovoid to pyriform cirrus sac, three to five testes, external seminal vesicle present and separated from cirrus sac by long seminal duct surrounded by glandular material, uterus ephemeral, eggs forming rapidly in gravid segments, and seminal receptacle present. *Pritchardia boliviensis* n. sp. includes a single species that occurs in small marsupials in the family Didelphidae of the lowlands and Yungas of Bolivia and Atlantic forests of Paraguay.

Key words: Bolivia, cestode, Didelphidae, helminth, marsupial, Paraguay, *Pritchardia boliviensis*, Yungas

INTRODUCTION

Bolivia is a country of massive biological diversity situated at the juncture of tropical, temperate, mountainous, and lowland zones of the south-central Neotropical region. Within the geopolitical boundaries of Bolivia more than 327 species of mammals have been documented, of which more than 5% are endemic to the country (Anderson 1997). Prior work by Gardner and Campbell (1992a) on endoparasites of mammals of Bolivia showed that several areas of Bolivia are unique in having very old ecological connections with Australian monotremes and marsupials. The following paper provides the description of a new species of

cestode that necessitates creation of a new genus in the family Anoplocephalidae.

Nine species of cestodes in the family Anoplocephalidae are currently known from marsupials (Didelphimorphia: Didelphidae) in the Neotropical and Nearctic zoogeographic regions including seven species now classified in the genus *Mathevotaenia* Akhumyan 1946 and two species in *Paralinstowia* Baer 1927 (see summaries in Gardner and Campbell 1992a; Campbell et al. 2003; Jiménez et al. 2008). Published results of surveys of mammals in these regions have

shown that these anoplocephalid cestodes have been frequently reported from three species of marsupials including *Didelphis virginiana* Kerr 1792 (the Virginia opossum), *Didelphis aurita* (Wied-Neuwied 1826) (the big-eared opossum), and *Philander opossum* (Linnaeus 1758) (the gray four-eyed opossum) (Sandars 1957; Gomes 1979; Campbell et al. 2003).

Few published records exist that report cestodes of the Anoplocephalidae as parasites of small-bodied marsupials in the Neotropical and southern Nearctic biogeographic regions, and at the present time, the only groups of these opossums that have been investigated, even superficially for their parasites, include species distributed among the genera *Monodelphis* Burnett 1830, *Thylamys* Gray 1843, and *Marmosa* Gray 1821 (see Gardner and Campbell 1992a; Campbell et al. 2003; Jiménez et al. 2008).

During field-work conducted throughout Bolivia including the years 1984 – 2000, the American

Museum of Natural History (AMNH), the Museum of Southwestern Biology (MSB), the Harold W. Manter Laboratory of Parasitology (HWML), and the Colección Boliviana de Fauna (CBF) of the Bolivian National Museum of Natural History in La Paz mounted joint collecting expeditions to survey and inventory sylvatic mammals and their parasites. A major part of the work on the taxonomy and distribution of the mammals themselves has been published by Anderson (1997) and many groups of parasites that were collected there are now being studied. From most of the mammals that were collected by these expeditionary research teams, data on habitat, habits, and biological associates were recorded and archived in museums.

Here we describe one new species and create a new genus for tapeworms found in small-sized opossums of the subfamily Didelphinae (Marsupialia: Didelphidae) collected from several localities in Bolivia and from a single collection instance from Paraguay.

MATERIALS AND METHODS

All mammals were collected using Sherman™ live traps baited with a mixture of oatmeal, vanilla, tuna, and sardines, or with snap traps baited with peanut butter. Traps were placed in suitable habitat each evening and checked at first daylight the following morning. Details of each mammal collected were recorded in a field-collection catalog book and in the trap data book, copies of which are maintained in the H. W. Manter Laboratory of Parasitology (HWML), University of Nebraska State Museum (University of Nebraska-Lincoln); the original field catalogs and field notes are filed in the Department of Mammalogy, the American Museum of Natural History (AMNH), New York. Data regarding mammals collected in Paraguay are archived in the Mammal Collection of the Museum of Texas Tech University (TTU), Lubbock, Texas. Additional details of trapping localities can be found in field notes of the expeditions that refer to specimens of mammals maintained at American Museum of Natural History, TTU, and the Field Museum (FMNH), Chicago. Data on the specimens of parasites are stored in the HWML. Mammal voucher specimens are deposited in the following institutions: AMNH, MSB, TTU, FMNH, and the Colección Boliviana de Fauna (CBF),

Sección Mastozoología, Museo Nacional de Historia Natural, La Paz, Bolivia.

Mammals collected and examined for parasites were processed as follows: all organs of the digestive system were removed, placed in a Petri dish, wetted with water or physiological saline, and each organ was examined separately with the aid of an Optivisor™ at or exceeding 4x magnification and (or) with dissecting microscope up to 20x magnification. Tapeworms found were placed in distilled water until they relaxed and were then killed and preserved in either 70% ethanol or fixed in hot or cold 10% aqueous v/v formalin. All worms were transported and stored in the same medium in which they were preserved until they were studied. For future investigations of variation in DNA, some specimens, recovered before exposure to any fixative, were preserved in vials filled with 95% aqueous v/v EtOH, or placed in cryotubes, frozen in liquid nitrogen and stored at -85°C in an ultra-low freezer in the HWML (investigation of variation in molecules of DNA will be reported separately). Tapeworms were stained in Semichons' acetic carmine, dehydrated in an ethanol series, cleared in terpineol and xylene, and

mounted in damar gum on a glass microscope slide under a no. 1 cover slip. Drawings were made using a computer tablet and Adobe Photoshop CS5 with layering of line art over TIFF images (taken with a Zeiss AxioPhot microscope) in background layers which were then deleted before the final plate was prepared as a flat image. Images were captured and specimens were

measured and studied using either a Zeiss Ultraphot™ microscope and digital measuring software (SigmaScan Pro™, SPSS Science, Chicago, IL) or a Zeiss AxioPhot™ with Zeiss software. All measurements are given in micrometers. For each character, the range is given first, followed by mean, coefficient of variation, and sample size (Sokal and Rohlf 1995).

RESULTS

During our work in Bolivia, three white-bellied slender mouse opossums *Marmosops noctivagus* (Tschudi 1844), one brown four-eyed opossum *Metachirus nudicaudatus* (Geoffroy 1803), one Dorthoys' slender opossum *Marmosops dorthoys* (Thomas 1911), and one Gracile mouse opossum *Gracilinanus* sp. Gardner and Creighton 1989 were examined and found in-

fectured with anoplocephalid tapeworms. The specimens of *Marmosops* and *Metachirus* were collected in the lowlands and Yungas of Bolivia while the specimen of *Gracilinanus* was collected from remnant habitat in the Atlantic Forest of Paraguay. At the time of this writing, the specimen of *Gracilinanus* from Paraguay has not yet been identified (de la Sancha, pers comm.).

DESCRIPTION

Pritchardia n. gen.

Generic Diagnosis.—Anoplocephalidae, Linstowiinae. Strobilae very small, apolytic, with maximum of 3 acraspedote segments. First segment not conspicuously divided from scolex. Genital *Anlagen* visible in first developing segment, 2nd proglottid mature, 3rd proglottid gravid, longer than wide. Scolex with 4 unarmed suckers. Neck absent. Cirrus with rugose covering of minute spines. Vas deferens long and coiled, extending from external seminal vesicle to pyriform cirrus sac. External seminal vesicle present. Testes occupying medial part of segment mostly anterior to ovary. Ovary diffuse and reticulate, located posterior to cirrus sac in posterior 1/2 of proglottid generally on the poral side of the segment, never crossing osmoregulatory canals. Vitellarium diffuse, located near posterior margin of mature proglottid. Uterus ephemeral, forming egg capsules, each with a single egg. Vagina entering genital atrium dorsally, extending antiporad from atrium posteriad toward seminal receptacle. Seminal receptacle not overlapping cirrus sac and always anterior to or at same level as anteriormost ovarian follicles. Genital pores alternating regularly. Genital ducts cross excretory canals ventrally.

Taxonomic Summary

Etymology.—*Pritchardia* is named after Dr. Mary Lou Pritchard, former curator of the HWML Parasite Collection and director of the Harold W. Manter Laboratory of Parasitology, the University of Nebraska State Museum. Dr. Pritchard established the H. W. Manter Laboratory after the untimely death of Dr. Harold W. Manter in 1971. The generic name is used as feminine.

Diagnosis.—*Pritchardia* n. gen. is established to include species of cestodes that have an unarmed rostellum, unarmed suckers, suckers that are not covered in pockets of tissue and have compact vitellaria, a diffuse reticulate ovary, minute strobila, and eggs in capsules. The new genus is assigned to the Anoplocephalidae: Linstowiinae because eggs occur in capsules scattered throughout the medullary parenchyma of the gravid segment of each cestode; each egg capsule contains a single egg. In gravid segments, eggs are located only in the central medulla.

Species of *Pritchardia* can be recognized as distinct from other species included in the 13 genera of the Linstowiinae as follows: absence of lappets on suckers and single set of genitalia per segment while

species in *Tupaiaetaenia* Schmidt and File 1977 and *Panceriella* Stunkard 1969 are characterized by having lappets and double genitalia, respectively. The diffuse vitellarium located in the posterior part of the segment distinguishes species in the new genus from three other genera including *Linstowia* Zschokke 1899 and *Echidnotaenia* Beveridge 1980, in which the vitellarium is elongated, and from *Gekkotaenia* Bursey, Goldberg and Kraus 2005, which has both the vitellarium and ovary in a poral position. Testes in *Pritchardia* n. gen. are located anterior to the vitelline gland and lateral to the diffuse, reticulate ovary, contrasting with the antiporal testes in *Gekkotaenia* and with the arrangement of testes divided in two lateral groups in both *Cycloskrjabinia* Spasskii 1951 and *Witenbergitaenia* Wertheim, Schmidt and Greenberg 1986. *Pritchardia* n. sp. have testes lateral to and anterior to the ovary which contrasts to the arrangement in species of *Atriotaenia* Sandground 1926, *Oochoristica* Lühe 1898, and *Semenoviella* Spasskii 1951. In *Pritchardia* n. gen., all proglottids are acraspedote in contrast to the craspedote nature of *Atriotaenia*, *Witenbergitaenia*, *Paralinstowia* Baer 1927, and *Mathevotaenia* Akhmyan 1946. Lastly the genital ducts pass ventrally to the osmoregulatory canals while species of *Sinaiotaenia* Wertheim and Greenberg 1971 have genital ducts passing between osmoregulatory canals.

Pritchardia n. gen. shares several characters with species described in the genus *Paralinstowia*. However, species of *Pritchardia* have much smaller strobilae, fewer testes, a shallower genital atrium, ovoid to pyriform cirrus sac, well developed seminal receptacle, and an external seminal vesicle located at the extreme distal end of a convoluted seminal duct. In addition, the ovary in *Pritchardia* is reticulate in nature and the vitelline gland is diffuse this is in sharp contrast to the compact vitelline gland and tight bilobed ovaries of species of *Paralinstowia*. Finally, proglottids are acraspedote in *Pritchardia* and strongly craspedote in *Paralinstowia*.

***Pritchardia boliviensis* n. sp.**

(Fig. 1)



General (based on 38 whole mounts).—Strobilae small consisting of no more than three acraspedote proglottids. Total length 652 – 1,829 (1,304, 21%, n=30). Scolex unarmed, calyciform, conspicuously

wider than proglottids 192 – 397 (285, 18%, n=34) long, 227 – 479 (376, 17%, n=35) wide. Suckers oval, with complete, thick muscular walls 66 – 199 (147, 27%, n=134) long, 69 – 216 (155, 22%, n=136) wide. Suckers not able to retract into pockets and no tissue evident that would act as pockets for suckers. Neck absent. First proglottid not differentiated from scolex 49 – 415 (191, 66%, n=32) long, 37 – 409 (238, 50%, n=32) wide. Genital *Anlagen* visible in first segment and, depending on age of specimen, showing varying degrees of development, with different level of development in each specimen. Second proglottid, mature, 99 – 313 (189, 28%, n=35) long and 19 – 471 (261, 51%, n=34) wide. Third proglottid, gravid, 226 – 1,285 (789, 40%, n=27) long, 174 – 382 (265, 20%, n=27) wide. Through strobila, genital pores appear to alternate sides regularly. Genital pore located in anterior quarter of proglottid. Genital ducts crossing osmoregulatory canals ventrally.

Female reproductive system.—Ovary diffuse, reticulate, transversally elongated from medial part of segment toward proximal part of cirrus sac and situated posterior to cirrus sac and seminal receptacle. Mehlis' gland appears dorsal to vitelline gland. Vitelline gland located near distal part of segment, usually medial, appearing diffuse with duct exiting dorsad passing through Mehlis' gland traveling to join oviduct just distal to seminal receptacle. Vagina originating dorsally at genital pore about 5 μ m antiporal and directed mediad and expanding to form a seminal receptacle just past cirrus sac. Seminal receptacle provided with a single duct directed posteriad branching almost immediately with oviduct directed posteriad then turning antieriad toward ovary. Branch to uterus not seen. Uterus immediately developing single egg capsules in gravid segments. Eggs scattered through medulla of proglottid, rarely filling whole segment. Onchospheres 36 – 55 (44, 14%, n=19) in diameter. Egg 20 – 30 (23, 11%, n=19). Handle of embryonic hooks 5 – 8 (7, 13%, n=8) long and blade 3 – 5 (4, 16%, n=8) long (Fig. 1B).

Male reproductive system.—Three to five testes, lateral to and mostly anterior to reticulate ovary, visible in 2nd proglottid. Cirrus covered with minute rugose epithelium (arrow in Fig. 1C), convoluted within cirrus sac when invaginated. Cirrus sac ranging from almost circular to pyriform in shape; longitudinal axis of cirrus sac forming right angle with lateral margin of proglot-

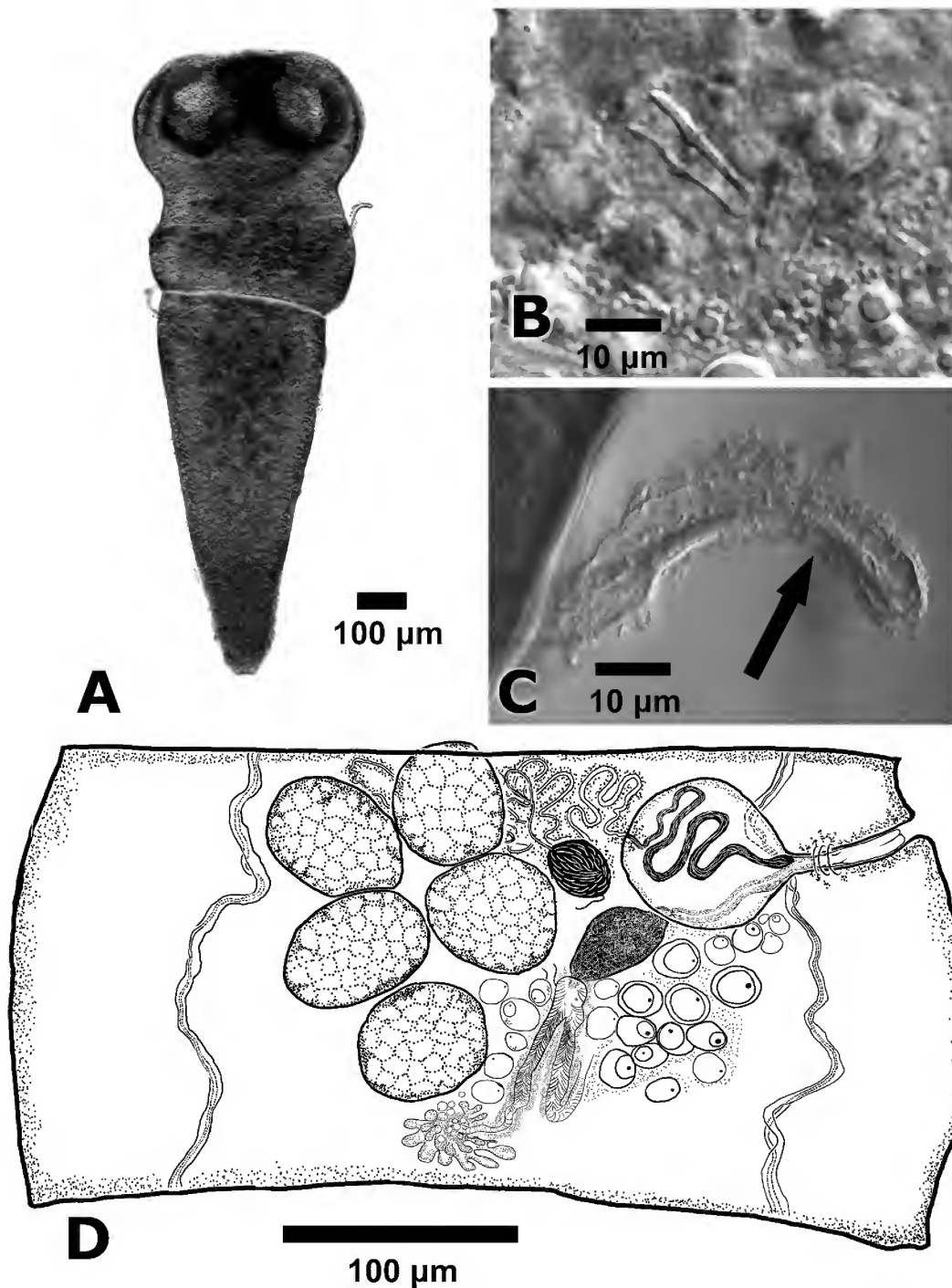


Figure 1. *Pritchardia boliviensis* n. gen. n. sp. A) Photomicrograph of complete specimen. Image shows scolex, Anlagen of genitalia in developing segment just posterior to scolex, mature proglottid, and terminal gravid proglottid. Scale = 100 μm. B) Embryonic hooks from egg capsule in uterus of gravid proglottid. Scale = 10 μm. C) Cirrus. Scale = 10 μm. D) Mature proglottid, showing arrangement of reproductive organs relative to osmoregulatory canals. Scale = 100 μm.

tid, in mature proglottids sac 60 – 130 (88, 17%, n=33) long, 41 – 59 (51, 8%, n=34) wide; duct 18 – 61 (27, 33%, n=31) long; in gravid proglottids sac 63 – 106 (85, 14%, n=24) long, 23 – 60 (51, 14%, n=24) wide, duct short 11 – 35 (24, 26%, n=25). Testes almost circular in outline 14–38 (28, 31%, n=8) by 17–38 (28, 27%, n=8). From testes, vasa efferentia connecting to a short vas deferens and then to a well developed external seminal vesicle which then connects via an exceptionally long and convoluted seminal duct, surrounded by glandular tissue. This duct then connects to the cirrus sac.

Taxonomic Summary

Type Host.—*Marmosops noctivagus* (Tschudi 1844) [white-bellied slender opossum].

Type Locality.—Bolivia: Cochabamba: 9.5 km by road NE of Tablas Monte, Rio Jatun Mayu; 17°02'29" S, 65°59'05" W (by GPS); 1500 m.

Symbiotype (see Frey et al. 1992).—*Marmosops noctivagus*, MSB catalog number MSB70278, Division of Biological Materials, New Mexico cryovoucher number (NK) NK30324. Collected on 14 July 1993.

Parasymbiotypes (from localities other than type locality and type host).—*Metachirus nudicaudatus* (Geoffroy 1803) [brown four-eyed opossum]; collection locality: Bolivia, La Paz, La Reserva: lat. 15°44' S, 67°31' W; 840 m. Colección Boliviana de Fauna (CBF) catalog number CBF2310, Division of Biological Materials, New Mexico cryovoucher number NK25551. Collected on 24 July 1992. *Gracilinanus* sp., collection locality: Paraguay: Alto Paraná: Estación Biológica Limoy (Parcela 2): lat. 24°43'52.1" S, long. 54°24'42.4" W; 230m. Collected on 15 March 2008. Texas Tech Museum number (TK) TK192462. *Marmosops dorthea* (Thomas 1911), collection locality: Bolivia: Santa Cruz, 3.5 km w. Pailón, estación on railroad: lat. 17°39' S, long. 62°45' W; 300 m. MSB55070, NK12286. Collected on 24 September 1984. *Marmosops noctivagus*, collection locality same as type locality. MSB catalog

number MSB70279, NK30340. Collected on 16 July 1993. Other specimens examined from hosts not in host parasymbiotype series: *Marmosops noctivagus*, collection locality same as type locality. MSB catalog number MSB140355, NK30327. Collected on 15 July 1993.

Parasite Specimens Deposited.—HOLOTYPE HWML49845, New Mexico cryovoucher number (host NK no.) NK30324, specimen labeled “a” on slide; PARATYPES (three specimens mounted on same slide as holotype from host no. NK30324), labeled as “c, d, e”. Additional paratype slides: HWML49271 (one slide from host no. Texas Tech (TK) TK129462), seven slides labeled as HWML49272 (from host no. TK129462), and four slides with 17 specimens (HWML49847 from host no. NK30324), 15 specimens on four slides labeled as HWML49848 from host no. NK30327, and 20 specimens on five slides labeled as HWML49849 from host no. NK30340. FIOCRUZ-CHIOC37318-19 (host nos. NK22551 and TK129462), UNAM-CNHE6422 (host no. NK25551), UNAM-CNHE6423 (host no. TK129462), and USNPC103071-72 (host no. NK25551 and no. TK129462).

Site of Infection in Host.—Anterior portion of small intestine, duodenum.

Etymology.—The species is named after the country of Bolivia from which the first specimens were discovered.

Remarks.—*Pritchardia boliviensis* n. sp. can be separated from all other species of anoplocephalid cestodes known on the basis of the extremely small strobila, the acraspedote proglottids, small number of testes, a reticulate ovary, the presence of an external seminal vesicle separated from the cirrus sac by a convoluted glandular seminal duct, the presence of a well developed seminal receptacle, and an almost round cirrus sac. In addition, *P. boliviensis* has a reticulate ovary and a relatively diffuse vitellarium and both immature and mature proglottids (1st and 2nd) are wider than long.

DISCUSSION

Spasskii and Buga (2002) discussed the possibility that the Anoplocephalidae may be a polyphyletic assemblage of species that can be separated into at least four phylogenetically unrelated groups of tapeworms (Anoplocephalidae, Hymenolepididae, Catenotaeniidae, and Davainiidae). These authors reorganized the taxonomic groups of the anoplocephalids, basing their decisions on an analysis of characters that included reproductive anatomy including the presence or absence of a seminal receptacle. In addition, Spasskii and Buga (2002) used non phylogenetic – non character based data to support their classification, including host-range (species of hosts in which the parasite is found) and the actual geographical distribution of the parasites.

In the same work, Spasskii and Buga (2002) proposed resurrecting the genus *Opossumia* Spasskii 1951 (which has been considered a junior synonym of *Mathevotaenia*) and to include this genus in Inversinae Spassky 1987 (Cyclophyllidea: Davaineata: Linstowiioidea: Linstowiidae). The Inversinae would then include *Inversia* Spassky 1951, *Atriotaeia*, *Opposumia*, *Paralinstowia*, *Sinaiotaenia*, and *Vasoramia* Spassky 1987, most of which are included in Linstowiinae *sensu* Beveridge (1994). Because the two classifications (Beveridge 1994 and Spasskii and Buga 2002) are based on different interpretations of the same characters, we think that a detailed revision of this group of tapeworms based on a phylogenetic analysis is necessary.

Although minutely small cestodes such *P. boliviensis* described herein have not been previously reported in the literature, the probability is good that there are many more species of small bodied flatworms occurring as parasites of marsupials in the Neotropical and southern Nearctic regions. Additional field-collecting of small marsupials using the methods outlined in Gardner and Jiménez-Ruiz (2009) will expand our understanding of these apparently common tapeworms.

Phylogenetic relationships among *P. boliviensis* and other species of Linstowiid cestodes across the New World are actively being investigated and will be published elsewhere. Previous descriptive work on Linstowiid cestodes in South America by Gardner and Campbell (1992a) and subsequent phylogenetic analysis of a broader group of these cestodes based on morphology (Gardner and Campbell 1992b) revealed some interesting trans-Antarctic zoogeographic relationships, that, if nothing else, show the ancient nature of this host-parasite assemblage. Future work on these cestodes and their marsupial (Neotropics) and monotreme (Australian) hosts will provide valuable insight into the nature and history of biodiversity; a starting place for this work will be a full inventory of marsupials and their parasites from many habitat types in the Neotropics.

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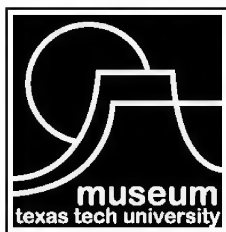
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